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COLON CANCER ANTIGEN PANEL

Field of the Invention

The invention relates to use of novel colon cancer-associated nucleic acid molecules and the polypeptides they encode as markers for cancer, including colon cancer. The invention also relates to the use of a panel of colon cancer-associated nucleic acid molecules and the polypeptides they encode and their use as markers for colon cancer. In addition, the invention relates to the use of such nucleic acid molecules and the polypeptides they encode for diagnosing colon cancer, and monitoring the colon cancer's response to treatment.

Background of the Invention

Colon cancer, which is also known as cancer of the large bowel and colorectal cancer, is second only to lung cancer as a cause of cancer death in the United States. Colorectal cancer is a common malignant condition that generally occurs in individuals 50 years of age or older; and the overall incidence rate of colon cancer has not changed substantially during the past 40 years. (Harrison's Principles of Internal Medicine, 14/e, McGraw-Hill Companies, New York, 1998). The treatment of colon cancer once diagnosis is made depends on the extent of the cancer's invasion of the colon tissue, lymph nodes, and metastasis to other organs such as the liver. The survival rate for patients diagnosed with early-stage cancer is about 90% survival after 5 years. The five-year survival rate drops if the cancer is not detected until the cancer has spread beyond the mucosal layer of the colon, and drops significantly further if, when detected, the cancer has spread beyond the colon to the lymph nodes and beyond. Thus, it is critical to diagnose colon cancer at the earliest possible stage to increase the likelihood of a positive prognosis and outcome.

The traditional method of colon cancer diagnosis is through the use of non-invasive or mildly invasive diagnostic tests, more invasive visual examination, and histologic examination of biopsy. Although these tests may detect colon cancers, each has drawbacks that limit its effectiveness as a diagnostic tool. One primary source of difficulty with most of the currently available methods for diagnosing colorectal cancer, is patient reluctance to submit to, or follow through with the procedures, due to the uncomfortable or perceived embarrassing nature of the tests.

Some of the less invasive diagnostic methods include fecal occult blood testing and digital rectal exam. A digital exam may detect tumors at the distal end of the colon/rectum,

but is not effective at more proximal levels. The usefulness of tests for occult blood is hampered by the intermittent bleeding patterns of colon cancers, which can result in a high percentage of false negative results. For example, approximately 50 percent of patients with documented colorectal cancers have a negative fecal blood test. In addition, false-positive fecal occult blood tests may also present problems for accurate diagnosis of colon cancer, because a number of non-colon cancer conditions (e.g.: gingivitis, ulcer, or aspirin use) may yield positive test results, resulting in unnecessary invasive follow-up procedures. These limitations of the less-invasive tests for colon cancer may delay a patient's procurement of rapid diagnosis and appropriate colon cancer treatment.

Visual examination of the colon for abnormalities can be performed through endoscopic or radiographic techniques such as rigid proctosigmoidoscopy, flexible sigmoidoscopy, colonoscopy, and barium-contrast enema. These methods are expensive, and uncomfortable, and also carry with them a risk of complications.

Another method of colon cancer diagnosis is the detection of carcinoembryonic antigen (CEA) in a blood sample from a subject, which when present at high levels, may indicate the presence of advanced colon cancer. But CEA levels may also be abnormally high when no cancer is present. Thus, this test is not selective for colon cancer, which limits the test's value as an accurate and reliable diagnostic tool. In addition, elevated CEA levels are not detectable until late-stage colon cancer, when the cure rate is low, treatment options limited, and patient prognosis poor.

More effective techniques for colon cancer diagnosis, and evaluation of colon cancer treatments are needed. Although available diagnostic procedures for colon cancer may be partially successful, the methods for detecting colon cancer remain unsatisfactory. There is a critical need for diagnostic tests that can detect colon cancer at its early stages, when appropriate treatment may substantially increase the likelihood of positive outcome for the patient.

Summary of the Invention

The invention provides methods for diagnosing colon cancer based on the identification of certain colon cancer-associated polypeptides and the encoding nucleic acid molecules thereof, as antigens that elicit immune responses in colon cancer. The identified antigens can be utilized as markers for diagnosing colon cancer, for following the course of treatment of colon cancer, and for assessing colon cancer treatments.

According to one aspect of the invention, methods for diagnosing colon cancer in a subject are provided. The methods include obtaining a biological sample from a subject, contacting the sample with at least two different colon cancer-associated polypeptides encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15, and determining specific binding between the colon cancer-associated polypeptides and agents in the sample, wherein the presence of specific binding is diagnostic for colon cancer in the subject.

According to another aspect of the invention, methods of determining onset, progression, or regression, of colon cancer in a subject are provided. The methods include obtaining from a subject a first biological sample, contacting the first sample with at least two different colon cancer-associated polypeptides encoded by nucleic acid molecules comprising a nucleotide sequence selected form the group consisting of SEQ ID NOs:1-15, determining specific binding between agents in the first sample and the at least two different colon cancer-associated polypeptides, obtaining from a subject a second biological sample, contacting the second biological sample with at least two different colon cancer-associated polypeptides encoded by nucleic acid molecules comprising a nucleotide sequence selected form the group consisting of SEQ ID NOs:1-15, determining specific binding between agents in the second sample and the at least two different colon cancer-associated polypeptides, and comparing the determination of binding in the first sample to the determination of specific binding in the second sample as a determination of the onset, progression, or regression of the colon cancer.

According to yet another aspect of the invention, methods for selecting a course of treatment of a subject having or suspected of having colon cancer is provided. The methods include obtaining from the subject a biological sample, contacting the sample with at least two different colon cancer-associated polypeptides encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15, determining specific binding between agents in the sample that are differentially expressed in different types of cancer, and the colon cancer-associated polypeptides, and selecting a course of treatment appropriate to the cancer of the subject. In some embodiments, the treatment is administering antibodies that specifically bind to the colon cancer-associated polypeptides. In some embodiments, the antibodies are labeled with one or more cytotoxic agents.

In some embodiments of the foregoing methods, the biological sample is a blood sample. In some embodiments, the agents are antibodies or antigen-binding fragments thereof. In some embodiments of the foregoing methods, the biological sample is contacted

with at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 different colon cancer-associated polypeptides encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:15. In some embodiments of the foregoing methods, the biological sample is contacted with a colon cancer-associated polypeptide other than those encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15.

According to another aspect of the invention, methods for diagnosing colon cancer in a subject are provided. The methods include obtaining a biological sample from a subject, contacting the sample with antibodies or antigen-binding fragments thereof, that bind specifically to at least two different colon cancer-associated polypeptides encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15, and determining specific binding between the antibodies or antigen-binding fragments thereof and colon cancer-associated polypeptides in the sample, wherein the presence of specific binding is diagnostic for colon cancer in the subject.

According to another aspect of the invention, methods for determining onset, progression, or regression, of colon cancer in a subject are provided. The methods include, obtaining from a subject a first biological sample, contacting the first sample with antibodies or antigen-binding fragments thereof, that bind specifically to at least two different colon cancer-associated polypeptides encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15, determining specific binding between colon cancer-associated polypeptides in the first sample and the antibodies or antigen-binding fragments thereof, obtaining from a subject a second biological sample, contacting the second sample with antibodies or antigen-binding fragments thereof, that bind specifically to at least two different colon cancer-associated polypeptides encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15, determining specific binding between colon cancer-associated polypeptides in the second sample and the antibodies or antigen-binding fragments thereof, and comparing the determination of specific binding in the first sample to the determination of specific binding in the second sample as a determination of the onset, progression, or regression of colon cancer.

According to another aspect of the invention methods for selecting a course of treatment of a subject having or suspected of having colon cancer are provided. The methods include obtaining from the subject a biological sample, contacting the sample with antibodies

or antigen-binding fragments thereof that bind specifically to at least two different colon cancer-associated polypeptides encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15, determining specific binding between colon cancer-associated polypeptides in the sample that are differentially expressed in different types of cancer, and the antibodies or antigen-binding fragments thereof, and selecting a course of treatment appropriate to the cancer of the subject. In some embodiments, the treatment is administering antibodies that specifically bind to the colon cancer-associated polypeptides. In some embodiments, the antibodies are labeled with one or more cytotoxic agents.

In some embodiments of the foregoing methods, the sample is selected from the group consisting of: tissue, stool, cells, blood, and mucus. In preferred embodiments of the foregoing methods, the tissue is colorectal tissue. In some embodiments of the foregoing methods, the antibodies are monoclonal or polyclonal antibodies, and in some embodiments, of the foregoing methods the antibodies are chimeric, human, or humanized antibodies. In some embodiments the antibodies are single chain antibodies, and in some embodiments of the foregoing methods, the antigen-binding fragments are $F(ab')_2$, Fab, Fd, or Fv fragments. In some embodiments of the foregoing methods, the biological sample is contacted with antibodies or antigen-binding fragments thereof, that bind specifically to at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 different colon cancer-associated polypeptides encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15. In some embodiments of the foregoing methods, the biological sample is contacted with an antibody or antigen-binding fragment thereof, that binds specifically to a colon cancer-associated polypeptide other than those encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15.

According to yet another aspect of the invention, kits for the diagnosis of colon cancer in a subject are provided. The kits include at least two different colon cancer-associated polypeptides encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of: SEQ ID NOs: 1-15, one or more control antigens, and instructions for the use of the polypeptides in the diagnosis of colon cancer. In some embodiments, the colon cancer-associated polypeptides are bound to a substrate. In some embodiments, the one or more agents are antibodies or antigen-binding fragments thereof. In some embodiments, the kit includes at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 different colon cancer-associated polypeptides encoded by nucleic acid molecules comprising

a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15. In some embodiments, the kit further includes a colon cancer-associated polypeptide other than those encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15.

According to yet another aspect of the invention, kits for the diagnosis of colon cancer in a subject are provided. The kits include antibodies or antigen-binding fragments thereof that bind specifically to at least two different colon cancer-associated polypeptides encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15, one or more control agents, and instructions for the use of the agents in the diagnosis of colon cancer. In some embodiments, the one or more agents are antibodies or antigen-binding fragments thereof. In some embodiments, the one or more agents are bound to a substrate. In some embodiments, the kit includes antibodies or antigen-binding fragments thereof, that bind specifically to least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 different colon cancer-associated polypeptides encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15. In some embodiments, the kit further includes an antibody or antigen-binding fragment thereof, that binds specifically to a colon cancer-associated polypeptide other than those encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15.

According to another aspect of the invention, protein microarrays are provided, which include at least two different colon cancer-associated polypeptides, wherein the colon cancer-associated polypeptides are encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of: SEQ ID NOs: 1-15, fixed to a solid substrate. In some embodiments, the microarray comprises at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 different colon cancer-associated polypeptides encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15. In some embodiments, the microarrays further consist essentially of a colon cancer-associated polypeptide other than those encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15. In some embodiments, microarray further consists essential of at least one control polypeptide molecule.

According to yet another aspect of the invention, protein microarrays are provided, which include antibodies or antigen-binding fragments thereof, that specifically bind at least two different colon cancer-associated polypeptides encoded by nucleic acid molecules

comprising a nucleotide sequence selected from the group consisting of: SEQ ID NOs:1-15, fixed to a solid substrate. In some embodiments, the protein microarray consists essentially of antibodies or antigen-binding fragments thereof, that bind specifically to least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 different colon cancer-associated polypeptides encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15. In some embodiments, the protein microarrays further consist essentially of an antibody or antigen-binding fragment thereof, that binds specifically to a colon cancer-associated polypeptide other than those encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15. In some embodiments, the protein microarrays further consist essentially of at least one control polypeptide molecule. In some embodiments, the antibodies are monoclonal or polyclonal antibodies. In some embodiments, the antibodies are chimeric, human, or humanized antibodies. In some embodiments, the antibodies are single chain antibodies, and in some embodiments, the antigen-binding fragments are $F(ab')_2$, Fab, Fd, or Fv fragments.

According to another aspect of the invention nucleic acid microarrays are provided. The nucleic acid microarrays include at least two nucleic acids selected from the group consisting of SEQ ID NOs: 1-15, fixed to a solid substrate. In some embodiments, the microarray consists essentially of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 different nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15. In some embodiments, the microarray further consists essentially of a nucleic acid molecule other than those selected from the group consisting of SEQ ID NOs:1-15. In yet another embodiment, the microarrays further consist essentially of at least one control nucleic acid molecule.

According to another aspect of the invention, methods for diagnosing colon cancer in a subject are provided. The methods include obtaining from the subject a biological sample, and determining the expression of at least two colon cancer-associated nucleic acid molecules or expression products thereof in the sample, wherein the nucleic acid molecules comprise a nucleotide sequence selected from the group consisting of: SEQ ID NO: 1-15, wherein the expression is diagnosis of the colon cancer in the subject. In some embodiments, expression is determined for at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15. In some embodiments, the method includes determining expression of a colon cancer-associated nucleic acid molecule other than those comprising a nucleotide sequence selected

from the group consisting of SEQ ID NOs:1-15. In some embodiments, the sample is selected from the group consisting of: tissue, stool, cells, blood, and mucus. In preferred embodiments, the tissue is colorectal tissue. In some embodiments, the expression of colon cancer-associated nucleic acid molecules is determined by a method selected from the group consisting of nucleic acid hybridization and nucleic acid amplification. In preferred embodiments, the hybridization is performed using a nucleic acid microarray.

According to yet another aspect of the invention, methods for determining onset, progression, or regression, of colon cancer in a subject are provided. The methods include obtaining from a subject a first biological sample, determining a level of expression of at least two colon cancer-associated nucleic acid molecules or expression products thereof in the first sample, wherein the nucleic acid molecules are selected from the group consisting of: SEQ ID NOs: 1-15, obtaining from the subject a second biological sample, determining a level of expression of at least two colon cancer-associated nucleic acid molecules or expression products thereof in the second sample, wherein the nucleic acid molecules are selected from the group consisting of: SEQ ID NOs: 1-15, and comparing the level of expression in the first sample to the level of expression in the second sample as a determination of the onset, progression, or regression of the colon cancer. In some embodiments, expression is determined for at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 nucleic acid molecules selected from the group consisting of SEQ ID NOs:1-15. In some embodiments, the method further includes determining expression for a colon cancer-associated nucleic acid molecule other than those comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15. In some embodiments, the sample is selected from the group consisting of: tissue, stool, cells, blood, and mucus. In preferred embodiments, the tissue is colorectal tissue. In some embodiments, the expression of colon cancer-associated nucleic acid molecules is determined by a method selected from the group consisting of nucleic acid hybridization and nucleic acid amplification. In preferred embodiments, the hybridization is performed using a nucleic acid microarray.

According to another aspect of the invention, methods for diagnosing cancer in a subject are provided. The methods include obtaining a biological sample from a subject, contacting the sample with a colon cancer-associated polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 2, 5, and 6, and determining specific binding between the colon cancer-associated

polypeptide and agents in the sample, wherein the presence of specific binding is diagnostic for cancer in the subject.

According to another aspect of the invention, methods for determining onset, progression, or regression, of cancer in a subject are provided. The methods include obtaining from a subject a first biological sample, contacting the first sample with a colon cancer associated polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 2, 5, and 6, determining specific binding between agents in the first sample and the colon cancer-associated, obtaining from a subject a second biological sample, contacting the second sample with a colon cancer associated polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 2, 5, and 6, determining specific binding between agents in the second sample and the colon cancer-associated polypeptide, and comparing the determination of binding in the first sample to the determination of specific binding in the second sample as a determination of the onset, progression, or regression of cancer.

According to another aspect of the invention, methods for selecting a course of treatment of a subject having or suspected of having cancer are provided. The methods include obtaining from the subject a biological sample, contacting the sample with a colon cancer-associated polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 2, 5, and 6, determining specific binding between agents in the sample that are differentially expressed in different types of cancer, and the colon cancer-associated polypeptide, and selecting a course of treatment appropriate to the cancer of the subject. In some embodiments, the treatment is administering antibodies that specifically bind to the colon cancer-associated polypeptide. In some embodiments, the antibodies are labeled with one or more cytotoxic agents.

In some embodiments of the foregoing methods, the sample is blood. In some embodiments of the foregoing methods, the agents are antibodies or antigen-binding fragments thereof. In preferred embodiments of the foregoing methods, the cancer is colon cancer.

According to another aspect of the invention, methods for diagnosing cancer in a subject are provided. The methods include obtaining a biological sample from a subject, contacting the sample with an antibody or antigen-binding fragment thereof, that binds specifically to a colon cancer-associated polypeptide encoded by a nucleic acid molecule

comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 2, 5, and 6, and determining specific binding between the antibody or antigen-binding fragment thereof and the colon cancer-associated polypeptide in the sample, wherein the presence of specific binding is diagnostic for cancer in the subject.

According to another aspect of the invention, methods for determining onset, progression, or regression, of cancer in a subject are provided. The methods include obtaining from a subject a first biological sample, contacting the first sample with antibodies or antigen-binding fragments thereof, that bind specifically to a colon cancer-associated polypeptides encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 2, 5, and 6, determining specific binding between colon cancer-associated polypeptides in the first sample and the antibodies or antigen-fragments thereof, obtaining from a subject a second biological sample, contacting the second sample with antibodies or antigen-binding fragments thereof, that bind specifically to a colon cancer-associated polypeptides encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 2, 5, and 6, determining specific binding between colon cancer-associated polypeptides in the second sample and the antibodies or antigen-binding fragments thereof, and comparing the determination of specific binding in the first sample to the determination of specific binding in the second sample as a determination of the onset, progression, or regression of cancer.

According to another aspect of the invention, methods for selecting a course of treatment of a subject having or suspected of having cancer are provided. The methods include obtaining from the subject a biological sample, contacting the sample with antibodies or antigen-binding fragments thereof that bind specifically to a colon cancer-associated polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 2, 5, and 6, determining specific binding between colon cancer-associated polypeptides in the sample that are differentially expressed in different types of cancer, and the antibodies or antigen-binding fragments thereof, and selecting a course of treatment appropriate to the cancer of the subject. In some embodiments, the treatment is administering antibodies that specifically bind to the colon cancer-associated polypeptide. In some embodiments, the antibodies are labeled with one or more cytotoxic agents.

In some embodiments of the foregoing methods, the sample is selected from the group consisting of: tissue, stool, cells, blood, and mucus. In some embodiments of the foregoing

methods, the tissue is colorectal tissue. In preferred embodiments of the foregoing methods, the antibodies are monoclonal or polyclonal antibodies, chimeric, human, or humanized antibodies. In some embodiments of the foregoing methods, the antibodies are single chain antibodies or antigen-binding fragments are $F(ab')_2$, Fab, Fd, or Fv fragments. In preferred embodiments of the foregoing methods, the cancer is colon cancer.

According to another aspect of the invention, kits for the diagnosis of cancer in a subject are provided. The kits include a colon cancer-associated polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of: SEQ ID NOs: 1, 2, 5, and 6; one or more control antigens; and instructions for the use of the polypeptide and control antigens in the diagnosis of cancer. In some embodiments, the colon cancer-associated polypeptide is bound to a substrate. In some embodiments, the one or more agents are antibodies or antigen-binding fragments thereof. In preferred embodiments, the cancer is colon cancer.

According to another aspect of the invention, kits for the diagnosis of cancer in a subject, are provided. The kits include antibodies or antigen-binding fragments thereof that bind specifically to a colon cancer-associated polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 2, 5, and 6; one or more control agents; and instructions for the use of the antibodies, antigen-binding fragments, and agents in the diagnosis of cancer. In some embodiments, the one or more agents are antibodies or antigen-binding fragments thereof. In some embodiments, the one or more agents are bound to a substrate. In preferred embodiments, the cancer is colon cancer.

According to another aspect of the invention, protein microarrays are provided. The protein microarrays include a colon cancer-associated polypeptide, wherein the colon cancer-associated polypeptide is encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of: SEQ ID NOs: 1, 2, 5, and 6, fixed to a solid substrate. In some embodiments, the protein microarray further includes at least one control polypeptide molecule.

According to yet another aspect of the invention, protein microarrays are provided. The protein microarrays include antibodies or antigen-binding fragments thereof, that specifically bind a colon cancer-associated polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of: SEQ ID NOs:1, 2, 5, and 6, fixed to a solid substrate. In some embodiments, the protein microarrays further

include at least one control polypeptide molecule. In some embodiments, the antibodies are monoclonal or polyclonal antibodies. In some embodiments, the antibodies are chimeric, human, or humanized antibodies and in some embodiments, the antibodies are single chain antibodies. In some embodiments, the antigen-binding fragments are $F(ab')_2$, Fab, Fd, or Fv fragments.

According to another aspect of the invention, nucleic acid microarrays are provided. The nucleic acid microarrays include a nucleic acid selected from the group consisting of SEQ ID NOs: 1, 2, 5, and 6, fixed to a solid substrate. In some embodiments, the nucleic acid microarrays further include at least one control nucleic acid molecule.

According to yet another aspect of the invention, methods for diagnosing cancer in a subject are provided. The methods include obtaining from the subject a biological sample, and determining the expression of a colon cancer-associated nucleic acid molecule or expression product thereof in the sample, wherein the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO: 1, 2, 5, and 6, wherein the expression is diagnostic of cancer in the subject. In some embodiments, the sample is selected from the group consisting of: tissue, stool, cells, blood, and mucus. In preferred embodiments, the tissue is colorectal tissue. In some embodiments, the expression of colon cancer-associated nucleic acid molecules is determined by a method selected from the group consisting of nucleic acid hybridization and nucleic acid amplification. In preferred embodiments, the hybridization is performed using a nucleic acid microarray. In preferred embodiments, the cancer is colon cancer.

According to another aspect of the invention, methods for determining onset, progression, or regression, of cancer in a subject are provided. The methods include obtaining from a subject a first biological sample, determining a level of expression of a colon cancer-associated nucleic acid molecule or expression products thereof in the first sample, wherein the nucleic acid molecule is selected from the group consisting of: SEQ ID NOs: 1, 2, 5, and 6, obtaining from the subject a second biological sample, determining a level of expression of a colon cancer-associated nucleic acid molecule or expression product thereof in the second sample, wherein the nucleic acid molecule is selected from the group consisting of: SEQ ID NOs: 1, 2, 5, and 6, and comparing the level of expression in the first sample to the level of expression in the second sample as a determination of the onset, progression, or regression of the cancer. In some embodiments, the sample is selected from the group consisting of: tissue, stool, cells, blood, and mucus. In preferred embodiments, the

tissue is colorectal tissue. In some embodiments, the expression of colon cancer-associated nucleic acid molecules is determined by a method selected from the group consisting of nucleic acid hybridization and nucleic acid amplification. In some embodiments, the hybridization is performed using a nucleic acid microarray. In preferred embodiments, the cancer is colon cancer.

Detailed Description of the Invention

The invention described herein relates to the identification of polypeptides that elicit specific immune responses in subjects with cancer, particularly colon cancer, which is also known as large-bowel cancer and colorectal cancer. Colon cancer-associated polypeptides have been identified through SEREX screening of patients with cancer. The SEREX method (*se*rological analysis of antigens by *r*ecombinant *ex*pression cloning), has been described by Sahin et al. (*Proc. Natl. Acad. Sci. USA* 92:11810-11813, 1995). The newly identified colon cancer-associated polypeptides and the encoding nucleic acid molecules thereof may be used as markers for cancer, including colon cancer, and may be used in the diagnosis and treatment assessment of colon cancer in humans. In addition, sets of at least two colon cancer-associated polypeptides and the encoding nucleic acid molecules thereof, may be used as markers in the diagnosis and treatment assessment of colon cancer in humans.

Polypeptides that elicit specific immune responses in colon cancer have now been identified and this identification allows use of these newly identified colon cancer-associated polypeptides or the encoding nucleic acids molecules thereof in cancer diagnostic assays and kits. In addition, sets of at least two of these new or previously identified polypeptides or the encoding nucleic acid molecules thereof, may be used in colon cancer diagnostic assays and kits. Such assays and kits are useful to detect colon cancer in human subjects, and for staging the progression, regression, or onset of colon cancer in subjects. The methods and kits described herein may also be used to evaluate treatments for colon cancer.

As used herein, "colon cancer-associated polypeptides" means polypeptides that elicit specific immune responses in animals having colon cancer and thus, include colon cancer-associated antigens and fragments of colon cancer-associated antigens, that are recognized by the immune system (e.g., by antibodies and/or T lymphocytes). The invention also relates to the use of the nucleic acid molecules that encode the colon cancer-associated polypeptides. In all embodiments, human colon cancer-associated polypeptides and the encoding nucleic acid molecules thereof, are preferred. As used herein, the "encoding

nucleic acid molecules thereof" means the nucleic acid molecules that code for the polypeptides.

As used herein, a subject is preferably a human, non-human primate, cow, horse, pig, sheep, goat, dog, cat, or rodent. In all embodiments, human subjects are preferred. In some embodiments, the subject is suspected of having cancer and in preferred embodiments the subject is suspected of having colon cancer. In some embodiments the subject has been diagnosed with cancer, and in preferred embodiments the subject has been diagnosed with colon cancer.

As used herein, "different types" of cancer may include different histological types, cell types, different stages of cancer, (e.g., primary tumor or metastatic growth).

Methods for identifying subjects suspected of having colon cancer may include fecal occult blood examination, digital examination, CEA testing, endoscopic or radiographic techniques, biopsy, subject's family medical history, subject's medical history, or imaging technologies, such as magnetic resonance imaging (MRI). Such methods for identifying subjects suspected of having colon cancer are well-known to those of skill in the medical arts. As used herein, a biological sample includes, but is not limited to: tissue, body fluid (e.g. blood), bodily exudate, mucus, and stool specimen. The tissue may be obtained from a subject or may be grown in culture (e.g. from a cell line).

As used herein, a colorectal tissue sample is tissue obtained (e.g., from a colorectal tissue biopsy) using methods well-known to those of ordinary skill in the related medical arts. The phrase "suspected of being cancerous" as used herein means a colon cancer tissue sample believed by one of ordinary skill in the medical arts to contain cancerous cells. Methods for obtaining the sample from the biopsy include gross apportioning of a mass, microdissection, laser-based microdissection, or other art-known cell-separation methods.

Because of the variability of the cell types in diseased-tissue biopsy material, and the variability in sensitivity of the diagnostic methods used, the sample size required for analysis may range from 1, 10, 50, 100, 200, 300, 500, 1000, 5000, 10,000, to 50,000 or more cells. The appropriate sample size may be determined based on the cellular composition and condition of the biopsy and the standard preparative steps for this determination and subsequent isolation of the nucleic acid for use in the invention are well known to one of ordinary skill in the art. An example of this, although not intended to be limiting, is that in some instances a sample from the biopsy may be sufficient for assessment of RNA expression without amplification, but in other instances the lack of suitable cells in a small

biopsy region may require use of RNA conversion and/or amplification methods or other methods to enhance resolution of the nucleic acid molecules. Such methods, which allow use of limited biopsy materials, are well known to those of ordinary skill in the art and include, but are not limited to: direct RNA amplification, reverse transcription of RNA to cDNA, amplification of cDNA, or the generation of radio-labeled nucleic acids.

In some embodiments, the colon cancer-associated nucleic acid molecules from the group of nucleic acid sequences numbered 1 through 15 in Table 3 (SEQ ID Nos: 1-15) and the colon cancer-associated polypeptides encoded by SEQ ID NOs: 1-15, are the group of polypeptide sequences SEQ ID NOs: 16 through 30 in Table 3. In some embodiments, colon cancer-associated polypeptides may include polypeptides other than those encoded by nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-15.

The invention involves in some embodiments, diagnosing or monitoring colon cancer in subjects by determining the presence of an immune response to at least two colon cancer-associated polypeptides. In some embodiments, cancer, such as colon cancer, in subjects may be diagnosed or monitored by determining the presence of an immune response to one of the novel colon cancer-associated polypeptides described herein. In preferred embodiments, this determination is performed by assaying a bodily fluid obtained from the subject, preferably blood, for the presence of antibodies against at least two colon cancer-associated polypeptides or the nucleic acid molecules that encode the cancer-associated polypeptides, or for the presence of antibodies against one of the novel colon cancer-associated polypeptides or the encoding nucleic acid molecules thereof as described herein. This determination may also be performed by assaying a tissue of the subject for the presence of at least two colon cancer-associated polypeptides and/or the encoding nucleic acid molecules thereof, or assaying a tissue of the subject for the presence of one of the novel colon cancer-associated polypeptides or the encoding nucleic acid molecules thereof as described herein.

Measurement of the immune response against one of the novel colon cancer-associated polypeptides described herein, or at least two colon cancer-associated polypeptides in a subject over time by sequential determinations permits monitoring of the disease and/or the effects of a course of treatment. For example, a sample may be obtained from a subject, tested for an immune response to one of the novel colon cancer-associated polypeptides or may be tested for an immune response to at least two colon cancer-associated polypeptides and at a second, subsequent time, another sample may be obtained from the subject and

similarly tested. The results of the first and second (subsequent) tests can be compared as a measure of the onset, regression or progression of colon cancer, or, if colon-cancer treatment was undertaken during the interval between obtaining the samples, the effectiveness of the treatment may be evaluated by comparing the results of the two tests.

The invention also involves in some embodiments diagnosing or monitoring colon cancer by determining the presence of at least two colon cancer-associated polypeptides and the encoding nucleic acid molecules thereof, or by determining the presence of one of the novel colon cancer-associated polypeptides and the encoding nucleic acid molecules thereof as described herein. In some important embodiments, this determination is performed by assaying a tissue sample from subject, preferably one believed to be cancerous, for the presence of at least two colon cancer-associated polypeptides or the encoding nucleic acid molecules thereof, or for the presence of one of the novel colon cancer-associated polypeptides and the encoding nucleic acid molecules thereof as described herein.

In other important embodiments, the presence of at least two colon cancer-associated polypeptides and the encoding nucleic acid molecules thereof, or the presence of one of the novel colon cancer-associated polypeptides and the encoding nucleic acid molecules thereof as described herein, are measured in mucus or fecal/stool samples. Such samples may contain colon cancer-associated polypeptides, or the encoding nucleic acids thereof, for example in shed cells. Measurement of the presence of at least two colon cancer-associated polypeptides and the encoding nucleic acid molecules thereof, or the presence of one of the novel colon cancer-associated polypeptides and the encoding nucleic acid molecules thereof as described herein, in subject's samples over time by sequential determinations at temporal intervals permits monitoring of the disease and/or the effects of a course of treatment.

In all embodiments, treatment for colon cancer may include, but is not limited to: surgical intervention, chemotherapy, radiotherapy, and adjuvant systemic therapies. In a preferred embodiment, treatment may include administering antibodies that specifically bind to the colon cancer-associated antigen. Optionally, an antibody can be linked to one or more detectable markers, antitumor agents or immunomodulators. Antitumor agents can include cytotoxic agents and agents that act on tumor neovasculature. Detectable markers include, for example, radioactive or fluorescent markers. Cytotoxic agents include cytotoxic radionuclides, chemical toxins and protein toxins.

The cytotoxic radionuclide or radiotherapeutic isotope may be an alpha-emitting isotope such as ^{225}Ac, ^{211}At, ^{212}Bi, or ^{213}Bi. Alternatively, the cytotoxic radionuclide may be a

beta-emitting isotope such as ^{186}Rh, ^{188}Rh, ^{90}Y, ^{131}I or ^{67}Cu. Further, the cytotoxic radionuclide may emit Auger and low energy electrons such as the isotopes ^{125}I, ^{123}I or ^{77}Br.

Suitable chemical toxins or chemotherapeutic agents include members of the enediyne family of molecules, such as chalicheamicin and esperamicin. Chemical toxins can also be taken from the group consisting of methotrexate, doxorubicin, melphalan, chlorambucil, ARA-C, vindesine, mitomycin C, cis-platinum, etoposide, bleomycin and 5-fluorouaracil. Other chemotherapeutic agents are known to those skilled in the art.

Agents that act on the tumor neovasculature can include tubulin-binding agents such as combrestatin A4 (Griggs et al., Lancet Oncol. 2:82, 2001) and angiostatin and endostatin (reviewed in Rosen, Oncologist 5:20, 2000, incorporated by reference herein). Immunomodulators may also be conjugated to colon cancer-associated antibodies.

The invention thus involves in one aspect, colon cancer-associated polypeptides, genes encoding those polypeptides, functional modifications and variants of the foregoing, useful fragments of the foregoing, as well as diagnostics relating thereto, and diagnostic uses thereof. In some embodiments, the colon cancer-associated polypeptide genes correspond to SEQ ID NOs: 1-15. Encoded polypeptides (e.g., proteins), peptides and antisera thereto are also preferred for diagnosis and correspond to SEQ ID NOs: 16-30. In some embodiments, encoded polypeptides (e.g. proteins), peptides, and antisera thereto are ones other than those corresponding to SEQ ID NOs:16-30.

Some of the amino acid sequences identified by SEREX as colon cancer-associated polypeptides, and the nucleotide sequences encoding them, are newly identified and some are sequences deposited in databases such as GenBank. The use of the newly identified sequences in diagnostic assays for cancer is novel, as is the use of sets of at least two or more of the sequences in colon cancer diagnostic assays and kits.

Homologs and alleles of the colon cancer-associated polypeptide nucleic acids of the invention can be identified by conventional techniques. Thus, an aspect of the invention is those nucleic acid sequences that code for colon cancer-associated antigens and antigenic fragments thereof. As used herein, a homolog to a colon cancer-associated polypeptide is a polypeptide from a human or other animal that has a high degree of structural similarity to the identified colon cancer-associated polypeptides.

Identification of human and other organism homologs of colon cancer-associated polypeptides will be familiar to those of skill in the art. In general, nucleic acid hybridization is a suitable method for identification of homologous sequences of another species (e.g.,

human, cow, sheep), which correspond to a known sequence. Standard nucleic acid hybridization procedures can be used to identify related nucleic acid sequences of selected percent identity. For example, one can construct a library of cDNAs reverse transcribed from the mRNA of a selected tissue (e.g., colon) and use the nucleic acids that encode colon cancer-associated polypeptide identified herein to screen the library for related nucleotide sequences. The screening preferably is performed using high-stringency conditions to identify those sequences that are closely related by sequence identity. Nucleic acids so identified can be translated into polypeptides and the polypeptides can be tested for activity.

The term "high stringency" as used herein refers to parameters with which the art is familiar. Nucleic acid hybridization parameters may be found in references that compile such methods, e.g. *Molecular Cloning: A Laboratory Manual*, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. More specifically, high-stringency conditions, as used herein, refers, for example, to hybridization at 65°C in hybridization buffer (3.5X SSC, 0.02% Ficoll, 0.02% polyvinyl pyrrolidone, 0.02% Bovine Serum Albumin, 2.5mM NaH_2PO_4(pH7), 0.5% SDS, 2mM EDTA). SSC is 0.15M sodium chloride/0.015M sodium citrate, pH7; SDS is sodium dodecyl sulphate; and EDTA is ethylenediaminetetracetic acid. After hybridization, the membrane upon which the DNA is transferred is washed, for example, in 2X SSC at room temperature and then at 0.1 - 0.5X SSC/0.1X SDS at temperatures up to 68°C.

There are other conditions, reagents, and so forth that can be used, which result in a similar degree of stringency. The skilled artisan will be familiar with such conditions, and thus they are not given here. It will be understood, however, that the skilled artisan will be able to manipulate the conditions in a manner to permit the clear identification of homologs and alleles of colon cancer-associated polypeptide nucleic acids of the invention (e.g., by using lower stringency conditions). The skilled artisan also is familiar with the methodology for screening cells and libraries for expression of such molecules, which then are routinely isolated, followed by isolation of the pertinent nucleic acid molecule and sequencing.

In general homologs and alleles typically will share at least 75% nucleotide identity and/or at least 90% amino acid identity to the sequences of colon cancer-associated antigen, antigenic fragment thereof, and antigen precursor thereof nucleic acid and polypeptides, respectively, in some instances will share at least 90% nucleotide identity and/or at least 95% amino acid identity, and in other instances will share at least 95% nucleotide identity and/or

at least 99% amino acid identity. The homology can be calculated using various, publicly available software tools developed by NCBI (Bethesda, Maryland) that can be obtained through the internet. Exemplary tools include the BLAST system available from the website of the National Center for Biotechnology Information (NCBI) at the National Institutes of Health. Pairwise and ClustalW alignments (BLOSUM30 matrix setting) as well as Kyte-Doolittle hydropathic analysis can be obtained using the MacVector sequence analysis software (Oxford Molecular Group). Watson-Crick complements of the foregoing nucleic acids also are embraced by the invention.

In screening for colon cancer-associated polypeptide genes, a Southern blot may be performed using the foregoing conditions, together with a detectably labeled probe (e.g. radioactive or chemiluminescent probes). After washing the membrane to which the DNA is finally transferred, the membrane can be placed against X-ray film or a phosphorimager to detect the radioactive or chemiluminescent signal. In screening for the expression of colon cancer-associated polypeptide nucleic acids, Northern blot hybridizations using the foregoing conditions can be performed on samples taken from colon cancer patients or subjects suspected of having a condition characterized by abnormal cell proliferation or neoplasia of the colorectal tissues. Amplification protocols such as polymerase chain reaction using primers that hybridize to the sequences presented also can be used for detection of the colon cancer-associated polypeptide genes or expression thereof.

Identification of related sequences can also be achieved using polymerase chain reaction (PCR) and other amplification techniques suitable for cloning related nucleic acid sequences. Preferably, PCR primers are selected to amplify portions of a nucleic acid sequence believed to be conserved (e.g., a catalytic domain, a DNA-binding domain, etc.). Again, nucleic acids are preferably amplified from a tissue-specific library (e.g., colon). One also can use expression cloning utilizing the antisera described herein to identify nucleic acids that encode related antigenic proteins in humans or other species using the SEREX procedure to screen the appropriate expression libraries. (See: Sahin et al. *Proc. Natl. Acad. Sci. USA* 92:11810-11813, 1995).

The invention also includes degenerate nucleic acids that include alternative codons to those present in the native materials. For example, serine residues are encoded by the codons TCA, AGT, TCC, TCG, TCT and AGC. Each of the six codons is equivalent for the purposes of encoding a serine residue. Thus, it will be apparent to one of ordinary skill in the art that any of the serine-encoding nucleotide triplets may be employed to direct the protein

synthesis apparatus, *in vitro* or *in vivo*, to incorporate a serine residue into an elongating colon cancer-associated polypeptide. Similarly, nucleotide sequence triplets which encode other amino acid residues include, but are not limited to: CCA, CCC, CCG, and CCT (proline codons); CGA, CGC, CGG, CGT, AGA, and AGG (arginine codons); ACA, ACC, ACG, and ACT (threonine codons); AAC and AAT (asparagine codons); and ATA, ATC, and ATT (isoleucine codons). Other amino acid residues may be encoded similarly by multiple nucleotide sequences. Thus, the invention embraces degenerate nucleic acids that differ from the biologically isolated nucleic acids in codon sequence due to the degeneracy of the genetic code.

The invention also provides modified nucleic acid molecules, which include additions, substitutions and deletions of one or more nucleotides. In preferred embodiments, these modified nucleic acid molecules and/or the polypeptides they encode retain at least one activity or function of the unmodified nucleic acid molecule and/or the polypeptides, such as antigenicity, receptor binding, etc. In certain embodiments, the modified nucleic acid molecules encode modified polypeptides, preferably polypeptides having conservative amino acid substitutions as are described elsewhere herein. The modified nucleic acid molecules are structurally related to the unmodified nucleic acid molecules and in preferred embodiments are sufficiently structurally related to the unmodified nucleic acid molecules so that the modified and unmodified nucleic acid molecules hybridize under stringent conditions known to one of skill in the art.

For example, modified nucleic acid molecules that encode polypeptides having single amino acid changes can be prepared. Each of these nucleic acid molecules can have one, two or three nucleotide substitutions exclusive of nucleotide changes corresponding to the degeneracy of the genetic code as described herein. Likewise, modified nucleic acid molecules that encode polypeptides having two amino acid changes can be prepared which have, e.g., 2-6 nucleotide changes. Numerous modified nucleic acid molecules like these will be readily envisioned by one of skill in the art, including for example, substitutions of nucleotides in codons encoding amino acids 2 and 3, 2 and 4, 2 and 5, 2 and 6, and so on. In the foregoing example, each combination of two amino acids is included in the set of modified nucleic acid molecules, as well as all nucleotide substitutions which code for the amino acid substitutions. Additional nucleic acid molecules that encode polypeptides having additional substitutions (i.e., 3 or more), additions or deletions (e.g., by introduction of a stop codon or a splice site(s)) also can be prepared and are embraced by the invention as readily

envisioned by one of ordinary skill in the art. Any of the foregoing nucleic acids or polypeptides can be tested by routine experimentation for retention of structural relation or activity to the nucleic acids and/or polypeptides disclosed herein.

The invention also provides nucleic acid molecules that encode antigenic fragments of colon cancer-associated proteins.

Fragments, can be used as probes in Southern and Northern blot assays to identify such nucleic acids, or can be used in amplification assays such as those employing PCR. As known to those skilled in the art, large probes such as 200, 250, 300 or more nucleotides are preferred for certain uses such as Southern and Northern blots, while smaller fragments will be preferred for uses such as PCR. Fragments also can be used to produce fusion proteins for generating antibodies or determining binding of the polypeptide fragments, or for generating immunoassay components. Likewise, fragments can be employed to produce nonfused fragments of the colon cancer-associated polypeptides, useful, for example, in the preparation of antibodies, and in immunoassays. Preferred fragments are antigenic fragments, which are recognized by agents that specifically bind to colon cancer-associated polypeptides. As used herein, colon cancer-associated antibodies, are antibodies that specifically bind to colon cancer-associated polypeptides.

The invention also permits the construction of colon cancer-associated polypeptide gene "knock-outs" or "knock-ins" in cells and in animals, providing materials for studying certain aspects of colon cancer and immune system responses to colon cancer by regulating the expression of colon cancer-associated polypeptides. For example, a knock-in mouse may be constructed and examined for clinical parallels between the model and a colon cancer-infected mouse with upregulated expression of a colon cancer-associated polypeptide, which may be useful to trigger an immune reaction to the polypeptide. Such a cellular or animal model may also be useful for assessing treatment strategies for colon cancer.

Alternative types of animal models for colon cancer may be developed based on the invention. Stimulating an immune response to a colon cancer-associated polypeptide in an animal may provide a model in which to test treatments, and assess the etiology of colon cancers.

The invention also provides isolated polypeptides (including whole proteins and partial proteins) encoded by the foregoing colon cancer-associated nucleic acids. Such polypeptides are useful, for example, alone or as fusion proteins to generate antibodies, and as components of an immunoassay or diagnostic assay. Colon cancer-associated

polypeptides can be isolated from biological samples including tissue or cell homogenates, and can also be expressed recombinantly in a variety of prokaryotic and eukaryotic expression systems by constructing an expression vector appropriate to the expression system, introducing the expression vector into the expression system, and isolating the recombinantly expressed protein. Short polypeptides, such as colon cancer-associated antigen fragments including antigenic peptides also can be synthesized chemically using well-established methods of peptide synthesis.

Fragments of a polypeptide preferably are those fragments that retain a distinct functional capability of the polypeptide. Functional capabilities that can be retained in a fragment of a polypeptide include interaction with antibodies (e.g. antigenic fragments), interaction with other polypeptides or fragments thereof, selective binding of nucleic acids or proteins, and enzymatic activity. One important activity is the ability to provoke in a subject an immune response. As will be recognized by those skilled in the art, the size of the fragment will depend upon factors such as whether the epitope recognized by an antibody is a linear epitope or a conformational epitope. Thus, some antigenic fragments of colon cancer-associated polypeptides will consist of longer segments while others will consist of shorter segments, (e.g. 5, 6, 7, 8, 9, 10, 11 or 12 or more amino acids long, including each integer up to the full length of the colon cancer-associated polypeptide). Those skilled in the art are well versed in methods for selecting antigenic fragments of proteins.

The skilled artisan will also realize that conservative amino acid substitutions may be made in colon cancer-associated polypeptides to provide functionally equivalent variants, or homologs of the foregoing polypeptides, i.e, the variants retain the functional capabilities of the colon cancer-associated antigen polypeptides. As used herein, a "conservative amino acid substitution" refers to an amino acid substitution that does not alter the relative charge or size characteristics of the protein in which the amino acid substitution is made. Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references that compile such methods, e.g. *Molecular Cloning: A Laboratory Manual*, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Exemplary functionally equivalent variants or homologs of the colon cancer-associated polypeptides include conservative amino acid substitutions of in the amino acid sequences of proteins disclosed herein. Conservative substitutions of amino acids include substitutions

made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D.

For example, upon determining that a peptide is a colon cancer-associated polypeptide, one can make conservative amino acid substitutions to the amino acid sequence of the peptide, and still have the polypeptide retain its specific antibody-binding characteristics.

Conservative amino-acid substitutions in the amino acid sequence of colon cancer-associated polypeptides to produce functionally equivalent variants of colon cancer-associated polypeptides typically are made by alteration of a nucleic acid encoding a colon cancer-associated polypeptide. Such substitutions can be made by a variety of methods known to one of ordinary skill in the art. For example, amino acid substitutions may be made by PCR-directed mutation, site-directed mutagenesis according to the method of Kunkel (Kunkel, *Proc. Nat. Acad. Sci. U.S.A.* 82: 488-492, 1985), or by chemical synthesis of a gene encoding a colon cancer-associated polypeptide. Where amino acid substitutions are made to a small unique fragment of a colon cancer-associated polypeptide, such as an antigenic epitope recognized by autologous or allogeneic sera or cytolytic T lymphocytes, the substitutions can be made by directly synthesizing the peptide. The activity of functionally equivalent fragments of colon cancer-associated polypeptides can be tested by cloning the gene encoding the altered colon cancer-associated polypeptide into a bacterial or mammalian expression vector, introducing the vector into an appropriate host cell, expressing the altered polypeptide, and testing for a functional capability of the colon cancer-associated polypeptides as disclosed herein. Peptides that are chemically synthesized can be tested directly for function, e.g., for binding to antisera recognizing associated antigens.

The invention as described herein has a number of uses, some of which are described elsewhere herein. First, the invention permits isolation of the colon cancer-associated protein molecules. A variety of methodologies well-known to the skilled practitioner can be utilized to obtain isolated colon cancer-associated polypeptide molecules. The polypeptide may be purified from cells that naturally produce the polypeptide, by chromatographic means or immunological recognition. Alternatively, an expression vector may be introduced into cells to cause production of the polypeptide. In another method, mRNA transcripts may be microinjected or otherwise introduced into cells to cause production of the encoded polypeptide. Translation of mRNA in cell-free extracts such as the reticulocyte lysate system also may be used to produce polypeptide. Those skilled in the art also can readily follow

known methods for isolating colon cancer-associated polypeptides. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography, and immune-affinity chromatography.

The isolation and identification of colon cancer-associated polypeptides also permits the artisan to diagnose a disorder characterized by expression of colon cancer-associated polypeptides, and characterized preferably by an immune response against the colon cancer-associated polypeptides.

The methods related to colon cancer-associated polypeptide immune responses involve determining the immune response (antibody or cellular) against one or more colon cancer-associated polypeptides. The immune response can be assayed by any of the various immunoassay methodologies known to one of ordinary skill in the art. For example, the antigenic colon cancer-associated polypeptides can be used as a target to capture antibodies from a blood sample drawn from a patient in an ELISA assay.

The methods related to colon cancer-associated polypeptide expression involve determining expression of one or more colon cancer-associated nucleic acids, and/or encoded colon cancer-associated polypeptides and/or peptides derived therefrom and comparing the expression with that in a colon cancer-free subject. Such determinations can be carried out via any standard nucleic acid determination assay, including the polymerase chain reaction, or assaying with labeled hybridization probes. Such hybridization methods include, but are not limited to microarray techniques.

The invention also makes it possible to isolate proteins that specifically bind to colon cancer-associated antigens as disclosed herein, including antibodies and cellular binding partners of the colon cancer-associated polypeptides. Additional uses are described further herein.

The invention also involves agents such as polypeptides that bind to colon cancer-associated polypeptides. Such binding agents can be used, for example, in screening assays to detect the presence or absence of colon cancer-associated polypeptides and complexes of colon cancer-associated polypeptides and their binding partners and in purification protocols to isolate colon cancer-associated polypeptides and complexes of colon cancer-associated polypeptides and their binding partners. Such agents also may be used to inhibit the native activity of the colon cancer-associated polypeptides, for example, by binding to such polypeptides.

The invention, therefore, embraces peptide binding agents which, for example, can be antibodies or fragments of antibodies having the ability to selectively bind to colon cancer-associated polypeptides. Antibodies include polyclonal and monoclonal antibodies, prepared according to conventional methodology.

Significantly, as is well-known in the art, only a small portion of an antibody molecule, the paratope, is involved in the binding of the antibody to its epitope (see, in general, Clark, W.R. (1986) The Experimental Foundations of Modern Immunology Wiley & Sons, Inc., New York; Roitt, I. (1991) Essential Immunology, 7th Ed., Blackwell Scientific Publications, Oxford). The pFc' and Fc regions, for example, are effectors of the complement cascade but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an $F(ab')_2$ fragment, retains both of the antigen binding sites of an intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule. Proceeding further, Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain denoted Fd. The Fd fragments are the major determinant of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

Within the antigen-binding portion of an antibody, as is well-known in the art, there are complementarity determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the paratope (see, in general, Clark, 1986; Roitt, 1991). In both the heavy chain Fd fragment and the light chain of IgG immunoglobulins, there are four framework regions (FR1 through FR4) separated respectively by three complementarity determining regions (CDR1 through CDR3). The CDRs, and in particular the CDR3 regions, and more particularly the heavy chain CDR3, are largely responsible for antibody specificity.

It is now well-established in the art that the non-CDR regions of a mammalian antibody may be replaced with similar regions of conspecific or heterospecific antibodies while retaining the epitopic specificity of the original antibody. This is most clearly manifested in the development and use of "humanized" antibodies in which non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody. See, e.g., U.S. patents 4,816,567, 5,225,539, 5,585,089, 5,693,762 and 5,859,205.

Fully human monoclonal antibodies also can be prepared by immunizing mice transgenic for large portions of human immunoglobulin heavy and light chain loci. Following immunization of these mice (e.g., XenoMouse (Abgenix), HuMAb mice (Medarex/GenPharm)), monoclonal antibodies can be prepared according to standard hybridoma technology. These monoclonal antibodies will have human immunoglobulin amino acid sequences and therefore will not provoke human anti-mouse antibody (HAMA) responses when administered to humans.

Thus, as will be apparent to one of ordinary skill in the art, the present invention also provides for $F(ab')_2$, Fab, Fv and Fd fragments; chimeric antibodies in which the Fc and/or FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric $F(ab')_2$ fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric Fab fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; and chimeric Fd fragment antibodies in which the FR and/or CDR1 and/or CDR2 regions have been replaced by homologous human or non-human sequences. The present invention also includes so-called single chain antibodies.

Thus, the invention involves polypeptides of numerous size and type that bind specifically to colon cancer-associated polypeptides, and complexes of both colon cancer-associated polypeptides and their binding partners. These polypeptides may be derived also from sources other than antibody technology. For example, such polypeptide binding agents can be provided by degenerate peptide libraries which can be readily prepared in solution, in immobilized form or as phage display libraries. Combinatorial libraries also can be synthesized of peptides containing one or more amino acids. Libraries further can be synthesized of peptoids and non-peptide synthetic moieties.

Phage display can be particularly effective in identifying binding peptides useful according to the invention. Briefly, one prepares a phage library (using e.g. m13, fd, or lambda phage), displaying inserts from 4 to about 80 amino acid residues using conventional procedures. The inserts may represent, for example, a completely degenerate or biased array. One then can select phage-bearing inserts which bind to the colon cancer-associated polypeptide. This process can be repeated through several cycles of reselection of phage that bind to the colon cancer-associated polypeptide. Repeated rounds lead to enrichment of phage bearing particular sequences. DNA sequence analysis can be conducted to identify the

sequences of the expressed polypeptides. The minimal linear portion of the sequence that binds to the colon cancer-associated polypeptide can be determined. One can repeat the procedure using a biased library containing inserts containing part or all of the minimal linear portion plus one or more additional degenerate residues upstream or downstream thereof. Yeast two-hybrid screening methods also may be used to identify polypeptides that bind to the colon cancer-associated polypeptides.

Thus, the colon cancer-associated polypeptides of the invention, including fragments thereof, can be used to screen peptide libraries, including phage display libraries, to identify and select peptide binding partners of the colon cancer-associated polypeptides of the invention. Such molecules can be used, as described, for screening assays, for purification protocols, for interfering directly with the functioning of colon cancer-associated polypeptides and for other purposes that will be apparent to those of ordinary skill in the art. For example, isolated colon cancer-associated polypeptides can be attached to a substrate (e.g., chromatographic media, such as polystyrene beads, or a filter), and then a solution suspected of containing the binding partner may be applied to the substrate. If a binding partner that can interact with colon cancer-associated polypeptides is present in the solution, then it will bind to the substrate-bound colon cancer-associated polypeptide. The binding partner then may be isolated.

As detailed herein, the foregoing antibodies and other binding molecules may be used for example, to identify tissues expressing protein or to purify protein. Antibodies also may be coupled to specific diagnostic labeling agents for imaging of cells and tissues that express colon cancer-associated polypeptides or to therapeutically useful agents according to standard coupling procedures. Diagnostic agents include, but are not limited to, barium sulfate, iocetamic acid, iopanoic acid, ipodate calcium, diatrizoate sodium, diatrizoate meglumine, metrizamide, tyropanoate sodium and radiodiagnostics including positron emitters such as fluorine-18 and carbon-11, gamma emitters such as iodine-123, technitium-99m, iodine-131 and indium-111, nuclides for nuclear magnetic resonance such as fluorine and gadolinium.

The invention also includes methods to monitor the onset, progression, or regression of colon cancer in a subject by, for example, obtaining samples at sequential times from a subject and assaying such samples for the presence and/or absence of an antigenic response that is a marker of the condition. A subject may be suspected of having colon cancer or may be believed not to have colon cancer and in the latter case, the sample may serve as a normal baseline level for comparison with subsequent samples.

Onset of a condition is the initiation of the changes associated with the condition in a subject. Such changes may be evidenced by physiological symptoms, or may be clinically asymptomatic. For example, the onset of colon cancer may be followed by a period during which there may be colon cancer-associated physiological changes in the subject, even though clinical symptoms may not be evident at that time. The progression of a condition follows onset and is the advancement of the physiological elements of the condition, which may or may not be marked by an increase in clinical symptoms. In contrast, the regression of a condition is a decrease in physiological characteristics of the condition, perhaps with a parallel reduction in symptoms, and may result from a treatment or may be a natural reversal in the condition.

A marker for colon cancer may be the specific binding of a colon cancer-associated polypeptide with an antibody. Onset of a colon cancer condition may be indicated by the appearance of such a marker(s) in a subject's samples where there was no such marker(s) determined previously. For example, if marker(s) for colon cancer are determined not to be present in a first sample from a subject, and colon cancer marker(s) are determined to be present in a second or subsequent sample from the subject, it may indicate the onset of cancer.

Progression and regression of a colon cancer condition may be generally indicated by the increase or decrease, respectively, of marker(s) in a subject's samples over time. For example, if marker(s) for colon cancer are determined to be present in a first sample from a subject and additional marker(s) or more of the initial marker(s) for colon cancer are determined to be present in a second or subsequent sample from the subject, it may indicate the progression of cancer. Regression of cancer may be indicated by finding that marker(s) determined to be present in a sample from a subject are not determined to be found, or found at lower amounts in a second or subsequent sample from the subject.

The progression and regression of a colon cancer condition may also be indicated based on characteristics of the colon cancer-associated polypeptides determined in the subject. For example, some colon cancer-associated polypeptides may be abnormally expressed at specific stages of colon cancer (e.g. early-stage colon cancer-associated polypeptides; mid-stage colon cancer-associated polypeptides; and late-stage colon cancer-associated polypeptides). Another example, although not intended to be limiting, is that colon cancer-associated polypeptides may be differentially expressed in primary tumors versus metastases, thereby allowing the stage and/or diagnostic level of the disease to be

established, based on the identification of selected colon cancer-associated polypeptides in a subject sample.

Another method of staging colon cancer may be based on variation in a subject's immune response to colon cancer-associated polypeptides, which may or may not be abnormally expressed in the subject. Variability in the immune response to the polypeptides may be used to indicate the stage of colon cancer in a subject, for example, some colon cancer-associated polypeptides may trigger an immune response at different stages of the colon cancer than that triggered by other colon cancer-associated polypeptides.

Different types of colon cancer, such as familial adenomatous polyposis (FAP) or hereditary nonpolyposis colon cancer (HNPCC), also known as Lynch syndrome, may express different colon cancer-associated polypeptides and the encoding nucleic acid molecules thereof, or may have different spatial or temporal expression patterns. Such variations may allow cancer-specific diagnosis and subsequent treatment tailored to the patient's specific condition. These colon cancer-specific diagnoses may also be based on the variations in immune responses to the different colon cancer-associated polypeptides.

The invention includes kits for assaying the presence of colon cancer-associated polypeptides and/or antibodies that specifically bind to colon cancer-associated polypeptides. An example of such a kit may include the above-mentioned polypeptides bound to a substrate, for example a dipstick, which is dipped into a blood or body fluid sample of a subject. The surface of the substrate may then be processed using procedures well known to those of skill in the art, to assess whether specific binding occurred between the polypeptides and agents (e.g. antibodies) in the subject's sample. For example, procedures may include, but are not limited to, contact with a secondary antibody, or other method that indicates the presence of specific binding.

Another example of a kit may include an antibody or antigen-binding fragment thereof, that binds specifically to a colon cancer-associated polypeptide. The antibody or antigen-binding fragment thereof, may be applied to a tissue sample from a patient with colon cancer and the sample then processed to assess whether specific binding occurs between the antibody and a polypeptide or other component of the sample. In addition, the antibody or antigen-binding fragment thereof, may be applied to a stool sample from a subject, either suspected of having colon cancer, diagnosed with colon cancer, or believed to be free of colon cancer. As will be understood by one of skill in the art, such binding assays may also be performed with a sample or object contacted with an antibody and/or colon cancer-

associated polypeptide that is in solution, for example in a 96-well plate or applied directly to an object surface.

The foregoing kits can include instructions or other printed material on how to use the various components of the kits for diagnostic purposes.

The invention further includes nucleic acid or protein microarrays with colon cancer-associated peptides or nucleic acids encoding such polypeptides. In this aspect of the invention, standard techniques of microarray technology are utilized to assess expression of the colon cancer-associated polypeptides and/or identify biological constituents that bind such polypeptides. The constituents of biological samples include antibodies, lymphocytes (particularly T lymphocytes), and the like. Protein microarray technology, which is also known by other names including: protein chip technology and solid-phase protein array technology, is well known to those of ordinary skill in the art and is based on, but not limited to, obtaining an array of identified peptides or proteins on a fixed substrate, binding target molecules or biological constituents to the peptides, and evaluating such binding. See, e.g., G. MacBeath and S.L. Schreiber, "Printing Proteins as Microarrays for High-Throughput Function Determination," *Science* 289(5485):1760-1763, 2000. Nucleic acid arrays, particularly arrays that bind colon cancer-associated peptides, also can be used for diagnostic applications, such as for identifying subjects that have a condition characterized by colon cancer-associated polypeptide expression.

Microarray substrates include but are not limited to glass, silica, aluminosilicates, borosilicates, metal oxides such as alumina and nickel oxide, various clays, nitrocellulose, or nylon. The microarray substrates may be coated with a compound to enhance synthesis of a probe (peptide or nucleic acid) on the substrate. Coupling agents or groups on the substrate can be used to covalently link the first nucleotide or amino acid to the substrate. A variety of coupling agents or groups are known to those of skill in the art. Peptide or nucleic acid probes thus can be synthesized directly on the substrate in a predetermined grid. Alternatively, peptide or nucleic acid probes can be spotted on the substrate, and in such cases the substrate may be coated with a compound to enhance binding of the probe to the substrate. In these embodiments, presynthesized probes are applied to the substrate in a precise, predetermined volume and grid pattern, preferably utilizing a computer-controlled robot to apply probe to the substrate in a contact-printing manner or in a non-contact manner such as ink jet or piezo-electric delivery. Probes may be covalently linked to the substrate.

Targets are peptides or proteins and may be natural or synthetic. The tissue may be obtained from a subject or may be grown in culture (e.g. from a cell line).

In some embodiments of the invention, one or more control peptide or protein molecules are attached to the substrate. Preferably, control peptide or protein molecules allow determination of factors such as peptide or protein quality and binding characteristics, reagent quality and effectiveness, hybridization success, and analysis thresholds and success.

Nucleic acid microarray technology, which is also known by other names including: DNA chip technology, gene chip technology, and solid-phase nucleic acid array technology, is well known to those of ordinary skill in the art and is based on, but not limited to, obtaining an array of identified nucleic acid probes on a fixed substrate, labeling target molecules with reporter molecules (e.g., radioactive, chemiluminescent, or fluorescent tags such as fluorescein, Cye3-dUTP, or Cye5-dUTP), hybridizing target nucleic acids to the probes, and evaluating target-probe hybridization. A probe with a nucleic acid sequence that perfectly matches the target sequence will, in general, result in detection of a stronger reporter-molecule signal than will probes with less perfect matches. Many components and techniques utilized in nucleic acid microarray technology are presented in *The Chipping Forecast*, Nature Genetics, Vol.21, Jan 1999, the entire contents of which is incorporated by reference herein.

According to the present invention, nucleic acid microarray substrates may include but are not limited to glass, silica, aluminosilicates, borosilicates, metal oxides such as alumina and nickel oxide, various clays, nitrocellulose, or nylon. In all embodiments, a glass substrate is preferred. According to the invention, probes are selected from the group of nucleic acids including, but not limited to: DNA, genomic DNA, cDNA, and oligonucleotides; and may be natural or synthetic. Oligonucleotide probes preferably are 20 to 25-mer oligonucleotides and DNA/cDNA probes preferably are 500 to 5000 bases in length, although other lengths may be used. Appropriate probe length may be determined by one of ordinary skill in the art by following art-known procedures. In one embodiment, preferred probes are sets of more than two of the colon cancer-associated polypeptide nucleic acid molecules set forth herein, or one of the novel colon cancer-associated polypeptide nucleic acid molecules as described herein. Probes may be purified to remove contaminants using standard methods known to those of ordinary skill in the art such as gel filtration or precipitation.

In one embodiment, the microarray substrate may be coated with a compound to enhance synthesis of the probe on the substrate. Such compounds include, but are not limited to, oligoethylene glycols. In another embodiment, coupling agents or groups on the substrate can be used to covalently link the first nucleotide or oligonucleotide to the substrate. These agents or groups may include, for example, amino, hydroxy, bromo, and carboxy groups. These reactive groups are preferably attached to the substrate through a hydrocarbyl radical such as an alkylene or phenylene divalent radical, one valence position occupied by the chain bonding and the remaining attached to the reactive groups. These hydrocarbyl groups may contain up to about ten carbon atoms, preferably up to about six carbon atoms. Alkylene radicals are usually preferred containing two to four carbon atoms in the principal chain. These and additional details of the process are disclosed, for example, in U.S. Patent 4,458,066, which is incorporated by reference in its entirety.

In one embodiment, probes are synthesized directly on the substrate in a predetermined grid pattern using methods such as light-directed chemical synthesis, photochemical deprotection, or delivery of nucleotide precursors to the substrate and subsequent probe production.

In another embodiment, the substrate may be coated with a compound to enhance binding of the probe to the substrate. Such compounds include, but are not limited to: polylysine, amino silanes, amino-reactive silanes (Chipping Forecast, 1999) or chromium. In this embodiment, presynthesized probes are applied to the substrate in a precise, predetermined volume and grid pattern, utilizing a computer-controlled robot to apply probe to the substrate in a contact-printing manner or in a non-contact manner such as ink jet or piezo-electric delivery. Probes may be covalently linked to the substrate with methods that include, but are not limited to, UV-irradiation. In another embodiment probes are linked to the substrate with heat.

Targets for microarrays are nucleic acids selected from the group, including but not limited to: DNA, genomic DNA, cDNA, RNA, mRNA and may be natural or synthetic. In all embodiments, nucleic acid target molecules from human tissue are preferred. The tissue may be obtained from a subject or may be grown in culture (e.g. from a cell line).

In embodiments of the invention one or more control nucleic acid molecules are attached to the substrate. Preferably, control nucleic acid molecules allow determination of factors such as nucleic acid quality and binding characteristics, reagent quality and effectiveness, hybridization success, and analysis thresholds and success. Control nucleic

acids may include but are not limited to expression products of genes such as housekeeping genes or fragments thereof.

In some embodiments, one or more control nucleic acid molecules are attached to the substrate. Preferably, control nucleic acid molecules allow determination of factors such as binding characteristics, reagent quality and effectiveness, hybridization success, and analysis thresholds and success.

Examples

Example 1

Method

Serum samples from patients with colon cancer were screened using a modification of the plaque assay, termed a spot assay. In this method, 80 x 120mm nitrocellulose membranes were precoated with a film of NZY/0.7% Agarose/2.5 mM IPTG and placed on a reservoir layer of NZY/0.7% Agarose in a 86 x 128mm Omni Tray (Nalge Nunc International Corp., Naperville, IL). Approximately 1.0 x 10^5 pfu of monoclonal phage encoding individual serologically defined colon cancer antigens, in a volume of 20μl, were mixed with 20μl of exponentially growing *E. coli* XL-1 Blue MRF and spotted (0.7-μl aliquots) on the precoated nitrocellulose membranes. Membranes were incubated for 15 hours at 37°C. A total of 75 different serologically defined colon cancer antigens were spotted in duplicate per nitrocellulose membrane. The agarose film was then removed from the membrane and the filters were processed for reactivity with individual serum samples (1:200 dilution), as described in Scanlan, et al., Int. J. Cancer *76*:652-658 (1998) and Scanlan, et al., Int. J. Cancer *83*:456-64, (1999).

Results

The results (see Table 1) indicate that 37/75 sera (49%) reacted with at least 1 antigen, 17/75 sera (23%) reacted with 2 or more antigens, 6/75 sera (8%) reacted with 3 or more antigens, and 2/75 sera (3%) reacted with 4 or more antigens. The reactivity of individual antigens is shown in Table 2.

Table 1. Colon Cancer Serology

Reactivity of 75 sera from colon cancer patients versus 15 antigens comprising, none of which react with normal sera (0/75, assayed by spot blot as described).

Sera Number	Reactive NY-antigens
COF1	Negative
COF2	Negative
COF3	Negative
COF4	Negative
COF5	Negative
COF6	CO61 +++
COF7	CO26 ++++, ESO-1 ++++, CO61 ++++
COF8	Negative
COF9	REN32 +++
COF10	p53 +++, CO58 ++
COF11	TNKL +, ESO-1 ++++
COF12	CO94 ++
COF13	Negative
COF14	Negative
COF15	SSX-2 ++
COF16	CO45 ++, CO42 ++
COF17	Negative
COF18	Negative
COF19	Negative
COF20	Negative
COF21	CO 58 +
COF22	TNKL ++, CO45 ++, CO42 ++
COF23	CO41 ++
CO24	Negative
CO25	Negative
CO26	TNKL +++
CO27	CO45 ++++
CO28	CO9 ++++, ESO-1 ++++, CO58 ++++, CO61 ++
CO29	MAGE-3 +, ESO-1 +
CO30	p53 +++
CO31	Negative
CO32	Negative
CO33	MAGE 3 +++
CO34	Negative
CO35	Negative
CO36	CO41 +++
CO37	Negative
CO38	Negative
CO39	Negative
CO40	CO42 +, CO95 +

Sera Number	Reactive NY-antigens
CO41	Negative
CO42	p53 ++++
CO43	p53 ++++, CO94 ++++
CO44	Negative
CO45	p53 +++
CO46	Negative
CO47	CO61 +
CO48	p53 ++++, MAGE 3 ++
CO49	Negative
CO50	Negative
CO51	CO9 +
COF52	Negative
CO53	TNKL +, p53 ++++
CO54	Negative
CO55	ESO-1 ++++
CO56	Negative
CO57	Negative
CO58	Negative
CO59	Negative
CO60	SSX-1 +, MAGE-3 +, CO42 +, CO61 ++++
CO61	TNKL ++
**CO62	**same sera as CO28
**CO63	**same sera as CO29
CO64	TNKL +
CO65	Negative
**CO66	**same sera as CO30
CO67	p53 ++
CO68	MAGE-3 +, CO42 +
CO69	Negative
CO70	Negative
CO71	REN32 +, MAGE-3 +
CO72	Negative
CO73	REN32 ++, p53 +
CO74	Negative
CO75	p53 +++
CO76	Negative
CO77	CO94 ++++, CO95 +++, p53 ++
CO78	CO42 ++, CO94 ++++, CO95 ++

Table 2: Reactivity of individual antigens (includes autologous where applicable)

CO13 (p53)	13/76
CO-26 (MNK 1):	2/76
ESO-1:	5/75
REN-32 (Lamin C):	3/75
TNKL (BC-203):	6/75
SSX-2:	2/75
CO-45 (Tudor like):	4/76
CO-41 (MBD2):	3/76
MAGE-3	6/75
CO-9 (HDAC 5)	3/76
CO-42 (TRIP4):	7/76
CO-61 (HIP1R):	5/75
CO-58 (KNSL6):	3/75
CO-94 (seb4D):	4/75
CO-95 (KIAA1416)	4/75

Table 3. Sequence Identification Numbers

Sequence Name	Nucleotide SEQ ID NO	Protein SEQ ID NO.
CO-95 (KIAA1416)	1	16
CO-94 (seb4D)	2	17
CO-9 (HDAC 5)	3	18
CO-61 (HIP1R)	4	19
CO-58 (KNSL6)	5	20
CO-45	6	21
CO-42 (TRIP4)	7	22
CO-41 (MBD2)	8	23
CO-13 (P53)	9	24
Ren-32 (Lamin C)	10	25
TNKL (BC-203)	11	26
CO-26 (MNK 1)	12	27
SSX-2	13	28
MAGE-3	14	29
ESO-1	15	30

Other aspects of the invention will be clear to the skilled artisan and need not be repeated here. Each reference cited herein is incorporated by reference in its entirety.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

We claim: